



Animal and Plant
Health Inspection
Service

Veterinary Services

Center for Veterinary
Biologics

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Leptospira canicola Challenge Culture - Lot ID IRP 653

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Section/Area: CVB-DAT

Effective Date: 24 May 2021

Notes: *Strain or Source: Moulton strain, NVSL No. 11203, Fill date: January 26, 2017*

**United States Department of Agriculture
Animal and Plant Health Inspection Service
Center for Veterinary Biologics
P. O. Box 844
Ames, IA 50010**

1. **Reagent Name:** *Leptospira canicola* Challenge Culture
2. **Strain or Source:** Moulton strain, NVSL No. 11203
3. **Lot Number:** IRP 653
4. **Fill Date:** January 26, 2017
5. **Expiration Date:** January 26, 2022

Precautions: *Leptospira* spp. are classified as biosafety level 2 pathogens. Handle under biological safety hood while wearing gloves. Use face shield when inoculating animals.

6. **Intended Use:** Challenge material for *L. canicola* testing.
7. **Instructions for Use:** Remove vials from cold storage and thaw at room temperature (18-27°C) for approximately one hour. A water bath or other warming device is not recommended. Do not vortex during thawing. An 18 gauge needle is recommended to remove cryopreserved challenge from the vial. You may draw the contents of more than one vial into a single syringe for convenience. Do not actively mix the contents of different vials to reduce stress on the spirochetes and reduce the human safety risk when working with a zoonotic agent.

Inoculate 50-90 g hamsters, preferably four hamsters total, with cryopreserved challenge using a 23 gauge or smaller bore needle. Each hamster should receive 1 mL IP. At least 50% of the inoculated hamsters should exhibit clinical signs within 10 days post-inoculation, and clinical signs within 4-5 days is expected. Hamster death prior to 3 days should not be attributed to leptospirosis. The *Leptospira* must be serially transferred through hamsters a minimum of three times before used as challenge material in a potency test according to Supplemental Assay Method (SAM) 609.
8. **Test of Reagent:** Identity was confirmed by ELISA, MAT, REA, PFGE, and MLST analysis. IRP 653 was verified as a virulent challenge when used in potency tests conducted according to SAM 609. The culture was negative for extraneous bacteria when incubated on blood agar plates at 37°C after three passages in hamsters. No hemadsorbing or cytopathogenic viruses were present based on bovine, canine, and hamster cells testing. Fluorescent antibody staining verified the absence of the following: CCV, CPV, CDV, REO 1, Rabies, BVDV1, BVDV2, BPV, BTV, BAV1, BAV5 and BRSV. PCR also verified the absence of BVDV1, BVDV2, PCV1, PCV2, and PRRS.

- 9. Container Size, Type, Weight, or Volume:** 2.0 mL cryovial containing 1.25 mL hamster liver homogenate with viable *L. canicola*.
- 10. Storage Conditions:** For long-term storage, challenge should be maintained in liquid nitrogen. Challenge may be stored at -80°C for storage less than one week.
- 11. CVB Technical Contact:** Bacteriology Section, Center for Veterinary Biologics, (515) 337-7020 or FAX (515) 337-7673.
- 12. Origin and Passage History:** Origin: Commercial laboratory in 1969. This strain is hamster virulent.
- 13. Method of Preparation:** Liver tissue from a *L. canicola* infected hamster is homogenized in liquid P80-BA medium with trimethoprin, sulfamethoxazole, and 5-fluorouracil and then frozen overnight at -80°C. Challenge is stored in liquid nitrogen until use or shipment.
- 14. Other:** None.

Reagent orders and feedback should be sent *including phone number* to the following email address: VS.DB.CVB.Reagent.Requests@usda.gov

Reagent orders forms (APHIS Form 2018) can be found on the CVB website.